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(54) Title: NEOMORPHOGENESIS OF UROLOGICAL STRUCTURES IN VIVO FROM CELL CULTURE

(57) Abstract

Methods and artificial matrices for the growth and implantation of urological structures and surfaces are disclosed in which urothelial cells are grown in culture on biodegradable, biocompatible, fibrous matrices formed of polymers, such as polyg-lycolic acid, polylactic acid, or other polymers which degrade over time. The cells can be cultured *in vitro* until an adequate cell volume and density has developed for the cells to survive and proliferate *in vivo*. Alternatively, when adequate cell numbers for implantation are available, the cells can be attached to the matrix and implanted directly, without proliferation *in vivo*. The implants approximate the desired urological structure to be replaced or repaired, such as the kidney, ureter, bladder, urethra, and the like. Implantation is followed by remodeling through cell growth and proliferation *in vivo*. In another aspect of the inventin, techniques are disclosed for selectively extracting or harvesting urothelial cells either from excised urological tissue *in vitro* or from intact urological tissue *in vivo* by treating the tissue with a digestive enzyme, such as collagenase.

NEOMORPHOGENESIS OF UROLOGICAL STRUCTURES IN VIVO
FROM CELL CULTURE

5   The United States Government has rights in
this invention pursuant Grant No. 6M 26698 awarded by
the National Institutes of Health.

Background of the Invention

10

        The technical field of this invention is
prosthetic surgery and cell culturing. In
particular, the invention pertains to methods and
implantable urological structures formed on

15 biocompatible artificial matrices for the
reconstruction or repair of urological structures.

        Shortage of native urothelium places
significant constraints on the success of surgical

20 reconstruction in a wide variety of urologic
conditions. Investigators have recognized this
problem and have looked for alternatives for
urothelial replacement. A variety of natural
tissues, such as omentum and seromuscular grafts, and

25 synthetic materials, such as polyvinyl sponge and
Teflon, have been tried in experimental and clinical
settings.

        Unfortunately, these attempts have not

30 produced satisfactory structural and functional
replacement. Synthetic materials alone cannot
replace selective transport functions of mucosal
tissue and commonly are lithogenic in the urinary
tract.

35

Bowel tissue has been used for urinary tract reconstruction for many years, however it is also associated with numerous complications. These include metabolic abnormalities, such as
5 hyperchloremic metabolic acidosis arising from electrolyte reabsorption, infection, perforation, stone formation, increased mucous production, diverticular formation, and malignancy. Despite these problems, incorporation of intestinal segments
10 into the urinary system has increased because of the lack of a suitable alternative.

A homologous substitute for urothelial tissues would be ideal. Recent work involving
15 hypospadias reconstruction using bladder mucosal grafts demonstrates the potential of autologous replacement. See, for example, Hendren and Reda, Vol. 21 J. Pediatric Surgery, pp. 181-192 (1986) and Ransley et al., Vol. 58 Brit. J. Urology, pp.331-333
20 (1986). However, the limited availability of urothelial tissue for patients requiring major reconstruction involving the kidney, ureter, bladder or urethra generally precludes its use.

25 The use of reconstructed sheets of autologous urothelium in urinary tract reconstruction would be ideal for a variety of surgical procedures and would have the added advantage of avoiding immunologic rejection. Autologous skin keratinocytes
30 have been used successfully in the treatment of

extensive burn wounds. See, for example, Green et
al., Vol. 76 <u>Proc. Nat'l. Acad. Sci</u>. pp. 5665-5668
(1979) and Burke et al., Vol. 194 <u>Ann. Surgery</u>,
pp. 413-428 (1981). Keratinocytes derived from the
5 urethral meatus have also been used in urethral
grafts for hypospadias repair in humans as described
in Romagnoli et al, Vol. 323 <u>New England J. medicine</u>,
pp. 527-530 (1990).

10   U.S. Serial No. 679,177 entitled "Chimeric
Neomorphogenesis Of Organs By Controlled Cellular
Implantation Using Artificial Matrices" filed
March 26, 1991, and U.S. Serial No. 933,018 entitled
"Chimeric Neomorphogenesis Of Organs Using Artificial
15 Matrices" filed November 20, 1986, by Joseph P.
Vacanti and Robert S. Langer, herein incorporated by
reference, describe methods and means whereby cells
having a desired function are grown on polymer
scaffolding using cell culture techniques, followed
20 by transfer of the cell polymer scaffold into a
patient at a site appropriate for attachment, growth
and function after attachment and equilibration to
produce a functional organ equivalent. Success
depends on the ability of the implanted cells to
25 attach to the surrounding environment and to
stimulate angiogenesis. Nutrients and growth factors
are supplied during cell culture allowing for
attachment, survival or growth as needed.

After the structure is implanted and growth
and vascularization take place, the resulting
organoid is a chimera formed of parenchymal elements
of the donated tissue and vascular and matrix
5 elements of the host. The polymer scaffolding used
for the initial cell culture is constructed of a
material which degrades over time and is, therefore,
not present in the chimeric organ. Vascular ingrowth
following implantation allows for normal feedback
10 mechanisms controlling the soluble products of the
implanted cells. The preferred material for forming
the matrix or support structure is a biodegradable,
artificial polymer, for example, polyglycolic acid,
polyorthoester or polyanhydride, which is degraded by
15 hydrolysis at a controlled rate and reabsorbed.

These materials provide the maximum control
of degradability, manageability, size and
configuration. In some embodiments, these materials
20 are overlaid with a second material, such as gelatin
or agarose, to enhance cell attachment. The polymer
matrix must be configured to provide both adequate
sites for attachment and adequate diffusion of
nutrients from the cell culture to maintain cell
25 viability and growth until the matrix is implanted
and vascularization has occurred. The preferred
structure for organ construction is a branched,
fibrous, tree-like structure formed of polymer fibers
having a high surface area which results in a
30 relatively shallow concentration gradient of
nutrients, wastes and gases so as to produce uniform
cell growth and proliferation.

U.S. Serial No. 933,018 and U. S. Serial No.
679,177 disclose several examples of the successful
culturing and implantation of hepatocytes, intestine,
and pancreas cells, with subsequent normal function,
5 including production and secretion of bioactive
molecules. Examples of such molecules include growth
hormone from pituitary cells, insulin and glycogen
from pancreatic cells and clotting factors from liver
cells. As described in these applications, however,
10 there is a need for a different type of functioning
"organ," one which provides primarily a structural
function. Examples of types of cells which are
useful in these applications include cartilage, bone
and muscle cells.
15

It is an object of the present invention to
provide a method and means for designing,
constructing and utilizing artificial matrices as
temporary scaffolding for cellular growth and
20 implantation of urological structures.

It is a further object of the invention to
provide biodegradable, non-toxic matrices which can
be utilized for cell growth, both in vitro and in
25 vivo, as supports for urological structures.

It is a still further object of the
invention to provide biodegradable, non-toxic
matrices which can be utilized for cell growth both
30 in vitro and in vivo, to replace or to repair
urological structures.

It is another object of this invention to
provide an in vitro system in which cells will retain
35 their normal morphology and cell function.

Summary of the Invention

Methods and artificial matrices for the growth and implantation of urological structures and
5 surfaces are disclosed.

In one aspect of the invention, urothelial cells are grown in culture on biodegradable, biocompatible, fibrous matrices formed of polymers,
10 such as polyglycolic acid, polylactic acid, or other polymers which degrade over time, as a function of hydrolysis of the polymer backbone into innocuous monomers. The matrices are designed to allow adequate nutrient and gas exchange to the cells until
15 engraftment and vascularization at the site of engraftment occurs. The cells can be cultured _in vitro_ until an adequate cell volume and density has developed for the cells to survive and proliferate _in vivo_, or maintained _in vitro_ for the purpose of
20 manufacturing bioactive molecules. Alternatively, when adequate cell numbers for implantation are available, the cells can be attached to the matrix and implanted directly, without proliferation _in vitro_. One advantage of the matrices is that they
25 can be cast or molded such that the cells, when seeded on them, approximate the desired urological structure to be replaced or repaired, such as the ureter, bladder, urethra, and the like. Implantation is followed by remodeling through cell growth and
30 proliferation _in vivo_.

In another aspect of the invention,
techniques are disclosed for establishing cultures of
transitional uroepithelial cells which can be
expanded _in vitro_ using explant culture techniques,
5 or _in vivo_ using graft techniques. For example, as a
first step in this approach, urothelial cells can be
disassociated into single cell preparations, placed
onto a synthetic substrate, and returned directly to
animals or humans, where the normal reparative
10 signals will direct regeneration, repair and
remodelling. In addition, this approach allows the
exploitation of the in vivo environment for the
enhancement of growth _in concert with_ the
preservation of differentiated function.
15

In yet another aspect of the invention,
techniques are disclosed for selectively extracting
or harvesting urothelial cells either from excised
urological tissue _in vitro_ or from intact urological
20 tissue _in vivo_ by treating the tissue with a
digestive enzyme, such as collagenase.

Detailed Description

        U.S. Serial No. 679,177 entitled Chimeric
Neomorphogenesis Of Organs By Controlled Cellular
5   Implantation Using Artificial Matrices filed
March 26, 1991, by Joseph P. Vacanti and Robert S.
Langer describes a technique of placing dispersed
cell types onto synthetic, biodegradable polymer
fibers in vitro which have been configured to produce
10 high cell densities by allowing adequate diffusion of
nutrients and waste as well as gas exchange. This
technique has been applied to urothelial cells for
the purpose of creating implants of urological
structures, such as the urethers, bladder, urethra
15 and the like.

        In the preferred method, polymer fibers are
placed in culture media containing urothelial cells,
where the urothelial cells attach to the fibers in
20 multiple layers and retain their normal
configuration, which appears to be essential for the
urothelial cells to maintain their normal function
and remain viable. This technique also allows
transplantation of the polymer cell scaffold into
25 animals without disrupting the complex of attached
urothelial cells. Transplantation of this complex
containing a high density of normally-functioning
urothelial cells with a large surface area into an
animal allows the cells to obtain adequate nutrition
30 by diffusion and successful engraftment of
functioning urothelial tissue, even in the initial
absence of vascularization.

The examples below demonstrate that it is
possible to grow in culture on fibers of
biodegradable polymers urothelial cells that appear
to be morphologically and functionally normal and
5 will proliferate to a cell density sufficient to
allow implantation of the cell polymer scaffold in
animals and successful engraftment with formation of
a new tissue equivalent as the polymer resorbs. The
examples also demonstrate that the polymer fiber
10 scaffold is essential in that neither injection of
free urothelial cells nor implantation of the polymer
fibers without attached urothelial cells results in
urological tissue formation.

15   The method and matrices providing structural
and functional urological equivalents using
bioabsorbable, artificial substrates as temporary
scaffolding for cellular transfer and implantation
reaffirms the principles first outlined in U.S.
20 Serial No. 933,018 filed November 20, 1986:

        1.   Every structure in living organisms is
in a dynamic state of equilibrium, undergoing
constant renewal, remodeling and replacement of
25 functional tissue which varies from organ to organ
and structure to structure.

        2.   Dissociated structural cells tend to
reform structure, depending on the environment in
30 which they are placed and the degree of alteration
which they have undergone.

3.   Cell shape is determined by
cytoskeletal components and attachment to matrix
plays an important role in cell division and
differentiated function. If dissociated cells are
5 placed into mature tissue as a suspension without
cell attachment, they may have difficulty finding
attachment sites, achieving polarity and functioning
because they begin without intrinsic organization.
This limits the total number of implanted cells which
10 can remain viable to organize, proliferate, and
function.

        The latter principle is a key point in the
configuration of the urothelial cell support
15 matrices. For an organ to be constructed in tissue
culture and subsequently successfully implanted, the
matrices must have sufficient surface area and
exposure to nutrients such that cellular growth and
differentiation can occur prior to the ingrowth of
20 blood vessels following implantation. After
implantation, the configuration must allow for
diffusion of nutrients and waste products and for
continued blood vessel ingrowth as cell proliferation
occurs.
25
        Urothelial cells are initially isolated and
cultured using a novel technique. Digestive enzymes
such as collagenase, trypsin or EDTA can be used to
isolate urothelial cells for recovery and seeding
30 purposes. In one approach, a urothelial biopsy
sample can be taken and the mucosa stripped away and
placed in an enzyme solution, such as an aqueous
collagenase solution having an enzyme concentration
from about 0.1 to about 0.75 percent. The urothelial

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cells will be liberated and can then be collected by centrifuging or the like. Alternatively, a catheter can be introduced into the bladder to fill the bladder with an enzyme solution (e.g., a milder

5 collagenase solution from about 0.05 to about 0.40 percent collagenase). Following irrigation of the bladder and collection of the rinses, urethelial cells can again be collected and used to seed a polymeric matrix.

10

Once the cells have begun to grow and cover the matrix, they are implanted in a patient at a site appropriate for attachment, growth and function. One of the advantages of a biodegradable polymeric matrix

15 is that angiogenic and other bioactive compounds can be incorporated directly into the matrix so that they are slowly released as the matrix degrades _in vivo_. As the cell polymer structure is vascularized and the structure degrades, the cells will differentiate

20 according to their inherent characteristics.

In the preferred embodiment, the matrix is formed of a bioabsorbable or biodegradable, synthetic polymer, such as a polyanhydride, polyorthoester or

25 polyglycolic acid. In some embodiments, attachment of the cells to the polymer is enhanced by coating the polymers with compounds, such as basement membrane components, agar, agarose, gelatin, gum arabic, collagens, fibronectin, laminin,

30 glycosaminoglycans, mixtures thereof, and other materials having properties similar to biological matrix molecules known to those skilled in the art of cell culture. All polymers must meet the mechanical and biochemical parameters necessary to provide

adequate support for the cells with subsequent growth
and proliferation. Factors, including nutrients,
growth factors, inducers of differentiation or
dedifferentiation, products of secretion,
5 immunomodulators, inhibitors of inflammation,
regression factors, biologically-active compounds
which enhance or allow ingrowth of the lymphatic
network or nerve fibers, and drugs, can be
incorporated into the matrix or provided in
10 conjunction with the matrix. Similarly, polymers
containing peptides, such as the attachment peptide
RGD (Arg-Gly-Asp) can be synthesized for use in
forming matrices.

15   A presently-preferred polymer is
Polyglactin, developed as absorbable synthetic suture
material, a 90:10 copolymer of glycolide and lactide,
manufactured as Vicryl® braided absorbable suture
(Ethicon Co., Somerville, New Jersey) See, Craig
20 et al.: A Biological Comparison Of Polyglactin 910
And Polyglycolic Acid Synthetic Absorbable Sutures.
Vol. 141, Surg., p. 1010 (1975). Polyglycolide
fibers can be used as supplied by the manufacturer.
Other shapes can be fabricated using one of the
25 following methods:

       Solvent Casting. A solution of polymer in
an appropriate solvent, such as methylene chloride,
is cast as a branching pattern relief structure.
30 After solvent evaporation, a thin film is obtained.

       Compression Molding. Polymer is pressed
(30,000 psi) into an appropriate pattern.

Filament Drawing. Filaments are drawn from the molten polymer.

Meshing. A mesh is formed by compressing
5 fibers into a felt-like material.

The polymers can be characterized with respect to mechanical properties, such as tensile strength using an Instron tester, for polymer
10 molecular weight by gel permeation chromatography (GPC), glass, transition temperature by differential scanning calorimetry (DSC) and bond structure by infrared (IR) spectroscopy; with respect to toxicology by initial screening tests involving Ames
15 assays and in vitro teratogenicity assays and implantation studies in animals for immunogenicity, inflammation, release and degradation studies. In vitro cell attachment and viability can be assessed using scanning electron microscopy, histology and
20 quantitative assessment with radioisotopes.

At the present time, a mesh-like structure formed of fibers, which may be round, scalloped, flattened, star shaped, solitary or entwined with
25 other fibers is preferred. The use of branching fibers is based upon the same principles which nature has used to solve the problem of increasing surface area proportionate to volume increases. All multicellular organisms utilize this repeating

branching structure. Branching systems represent
communication networks between organs, as well as the
functional units of individual organs. Seeding and
implanting this configuration with cells allows
5 implantation of large numbers of cells, each of which
is exposed to the environment of the host, providing
for free exchange of nutrients and waste while
neovascularization is achieved.

10   The polymeric matrix may be made flexible or
rigid, depending on the desired final form, structure
and function. For reconstruction of kidney
structures such as the renal pelvis as well as for
reconstruction of bladder, sheet-like matrices may be
15 preferable in many instances. For reconstruction of
the urethra and/or ureters, tubular matrices will
often be preferable.

        Cells may be derived from the host, a
20 related donor or from established cell lines. In one
variation of the method using a single matrix for
attachment of one or more cell lines, the scaffolding
is constructed, such that initial cell attachment and
growth occur separately within the matrix for each
25 population, for example, urothelial and
non-urothelial cell populations. Alternatively, a
unitary scaffolding may be formed of different
materials to optimize attachment of various types of
cells at specific locations. Attachment is a
30 function of both the type of cell and matrix
composition.

The following non-limiting examples
demonstrate actual attachment of cell preparations to
bioerodible, artificial polymers in cell culture and
implantation and engraftment of this polymer-cell
5 scaffold into animals. These samples describe
methods of harvesting primary uroepithelial cells
from rabbits and demonstrate the use of biodegradable
polyglycolic acid polymer matrices as vehicles for
delivery and maintenance of urothelium in host
10 animals to create new urothelial tissue _in_ _vitro_.

EXAMPLES

        Young adult New Zealand white rabbits
    (Charles River Labs, Wilmington, MA) were used as
 5 cell donors for all experiments. Young adult athymic
    nu/nu mice (Charles River Labs) were used as cell
    recipients. The animals were housed individually,
    allowed access to food and water ad lib, and
    maintained at 12 hr light and dark intervals.
10 Anesthesia for rabbits was carried out with an
    intramuscular injection of ketamine and rompum.
    Mice were anesthetized with Enfluorane by cone
    administration.

15   Biodegradable polymers were obtained as
    nonwoven meshes of polyglycolic acid, a synthetic
    polymer of hydroxyacetic acid (Davis and Geck,
    Danbury, CT). The average fiber diameter was 15 Mm.
    Interfiber distances varied between 0 and 200 Mm.
20 Dimensions of the polymer meshes used for
    implantation were 0.75 x 0.75 cm. Polymers were
    sterilized in ethylene oxide and sealed in aluminum
    foil until implantation.

25   Rabbit urothelial cells (RUC) for
    transplantation were isolated using the following
    method of cell harvesting. After the induction of
    anesthesia, the shaved abdomens of rabbits were
    painted with betadine and opened using sterile
30 technique. The bladders were isolated, the ureters
    ligated and the urethras cannulated. The bladders
    were rinsed with 3-5 ml of sterile phosphate buffered
    saline (PBS) and filled with a buffered collagenase
    solution comprised of 0.1-0.75% collagenase B from
35 Cl. histolyticum (Boehringer Mannheim, Indianapolis,
    IN) and sealed.

The ligated bladders were then placed in a
.50ml conical tube with the remaining volume filled
with PBS and incubated in a water bath at 37°C.
Incubation time was varied. Urothelial cell

5 suspensions were recovered from the donor bladders,
washed with a 4:1 mixture of DMEM and Ham's F12
medium (Gibco, Grant Island, NY) supplemented with 25
mg/ml adeninine, 0.244 mg/ml biotin, 0.0136 ng/ml
triiodothyronine, 5 mg/ml insulin, 5 mg/ml

10 transferrin, and penicillin and streptomycin, and
pelleted in a clinical centrifuge. Cell resuspension
and centrifugation was carried out an additional two
times.

15   The cells were then counted with a
hemacytometer and their viability determined by the
Trypan Blue exclusion method. The urothelial cells
were then seeded onto polyglycolic acid biodegradable
polymers and were maintained at 37'C in a humidified

20 atmosphere of 95%air/5% CO_2. After 1 to 4 days in
culture, the polymers were implanted into the
retroperitoneum, subcutaneous tissue or omentum of
athymic mice. Implants were then retrieved at
sacrifice at 5, 10, 20 and 30 days. Polymers

25 implanted without cells served as controls. Implants
were examined histologically and by western blot.

Implants recovered from host animals were
fixed in 10% neutral buffered formalin and embedded

30 in paraffin. Paraffin sections were stained with
hematoxylin and eosin.

Protein fractions enriched in cytoskeletal
proteins, according to protein isolation protocol
described in Freeman and Sueoka, Vol. 84, PNAS, pp.
5808-5812 (1987), were separated on 10%
5 polyacrylamide gels according to the procedure of
Laemmli. Approximately 15 mg protein was loaded per
lane. Proteins were electrophoretically blotted onto
Immunobilon-P blotting membranes (Millipore, Bedford,
MA). Non-specific sites were blocked in: 1% (w/v)
10 Carnation non-fat dry milk, 0.5% (v/v) Tween 20, 150
mM NaCl, 20 mM Tris base. AE1 and AE3 monoclonal
antibody mixture (Boehringer Mannheim) was incubated
at 2 mg/ml in blocking buffer for 2 hr at room
temperature.
15

     After repeated washes with blocking buffer,
the membrane was incubated for 2 hr with anti-mouse
IgG linked to alkaline phosphatase (Bio-Rad,
Richmond, CA) at 1:3000 dilution. Membranes were
20 washed repeatedly in blocking buffer, then in
substrate buffer (100M Tris base, 100mm NaCl, 50 MM
MgCl2, pH9.5) Colorimetric detection was with the
NBT/BCIP color reagent (Bio-Rad) until the bands were
adequately visualized.
25

     The objective of these experiments was to
determine whether newly harvested urothelial cells
would attach to biodegradable polymers in vitro and
whether these polymers would serve as a suitable
30 synthetic substrate for delivery and maintenance of
epithelial architecture in vivo. Explant techniques
have generally been used for the establishment of
primary cultures of uroepithelial cells. However,
for the experiments described here, the use of

explant cultures would not provide an adequate number
of cells. Therefore, a method was developed that
would allow isolation of bladder cell populations
efficiently, and in which large quantities of cells
5 enriched in uroepithelia can be obtained rapidly and
quantitatively. One approach was to excise
freshly-irrigated bladders from rodents and to treat
the mucosal surface with collagenase by incubating
the proteolytic enzyme solution within the enclosed,
10 ligated bladder for various times.

Initial attempts at cell harvest with this
method using Fisher rats resulted in the recovery of
less than 5×10^4 viable cells per bladder and,
15 although a limited sequence of implants into
synergetic hosts was carried out using these cells,
this approach was later abandoned. In subsequent
trials, in which bladders were obtained from New
Zealand white rabbits, adequate numbers of cells were
20 obtained reproducibly. Attempts to optimize the cell
harvest method by varying incubation time and
collagenase concentration resulted in establishment
of one preferred condition of incubation for 2 hr at
37°C in 0.5% collagenase. Assessment of cell
25 viability using the Trypan Blue exclusion method
indicated that these conditions resulted consistently
in the recovery of the greatest number of viable
cells. Quantitation of cell recovery by cell
counting over an extended period indicated that this
30 method resulted in an average recovery of 1.7×10^6 ±
1.01×10^5 viable cells/bladder (n=32).

Morphological analysis of these cell
populations plated onto plastic surfaces by phase
contrast microscopy indicated that they were composed
largely of uroepithelial cells, with very little
5 fibroblast contamination evident within the first
five days in culture. With maintenance of the
cultures for later times, fibroblast outgrowth
occurred. This result suggests that this method of
cell harvest results in recovery of populations
10 enriched in urothelium and that these populations can
be maintained as relatively homogeneous cultures at
least for several days.

Rabbit uroepithelial cells (RUC) obtained
15 using this cell harvest method were seeded directly
onto nonwoven polyglycolic polymers of approximately
0.56 cm^2 surface area in culture and allowed to
attach from between 1 to 4 days. Cell attachment to
the polymers occurred readily as assessed by phase
20 contrast microscopy with only limited numbers of
floating cells, indicating that under our conditions,
cultures enriched in healthy, viable cells were
obtained routinely. In most experiments polymer-cell
allografts were implanted into host animals after
25 incubation in culture medium overnight.

Allografts containing RUC were surgically
implanted in the mesentery, retroperitoneum, or
subcutaneous tissue of athymic (nude) mice (not
30 greater than three implants per animal) and recovered
at varying times after implantation. Twenty-eight
adult New Zealand white rabbits were used as donors

for cell harvest. Thirty-three nude mice were used
as recipients. Not including host animals lost by
attrition, a total of 58 cell-polymer scaffolds were
recovered from host animals at 5 to 30 day time
5 points. A total of 17 polymers implanted without
cells and maintained in vivo for the respective times
served as controls.

Histologic analysis demonstrated an
10 inflammatory response, which exhibited both an acute
phase and a chronic foreign body reaction.
Fibroblast infiltration and proliferation were seen
up to 10 days after implantation. Vascular ingrowth
was apparent in most implants by 5 days. Control
15 polymers elicited similar inflammatory and angiogenic
responses, suggesting that these responses were
largely due to the polymers.

Epithelial cells were evident in a
20 substantial fraction of RUC-polymer implants
recovered at each time point, including the
allografts maintained in vivo for 30 days.
Epithelial layers were frequently found associated
with polymer fibers. The number of implants
25 containing identifiable urothelial cells was highest
at the 5 day time point (89%). Single cell layers
were evident in 10 day implants and multiple
epithelial cell layers were evident in 20 day and 30
day implants. Recognizable epithelial populations
30 were not evident in any of the 17 control polymers.

These results indicate that RUC can survive
and can remain associated with the polymer fibers at
the ectopic implant sites even after extended periods
35 in vivo.

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Western blot analysis of protein fractures
obtained from 30 day RUC-polymer implants
demonstrated the presence of a prominently-expressed
40kDa cytokeratin present in rabbit bladder .
5 uroepithelium, identified using the AE1 and AE3
anticytokeratin monoclonal antibodies. In this
assay, this cytokeratin expression pattern can be
distinguished from that seen using protein from mouse
skin. Cytokeratins were not identifiable in protein
10 fractions from control polymers implanted without
cells. This result suggests the potential for
maintenance of uroepithelial differentiation in
implants maintained in host animals for long periods
at ectopic sites. This result also confirms the
15 histologic assessment that eosinophilic cell types
present in the polymer allografts at late time points
are RUC.

In summary, the feasibility of using
20 biodegradable polymers as delivery vehicles for
urothelial cell populations in vivo has been
demonstrated. Primary rabbit urothelial cells (RUC)
were found to attach readily to unwoven polyglycolic
acid polymers in vitro. Polymer-RUC allografts were
25 found to contain viable RUC after implantation times
for as long as 30 days at ectopic sites in athymic
mice, although some RUC cell death in the implants
was noted. Polymers were seen to evoke an angiogenic
response, which should allow RUC populations to
30 survive, and possibly proliferate, after extended
periods in vivo. RUC oriented themselves spatially
along the polymer surfaces and RUC populations
appeared to expand from one to several cell layers in
thickness with extended times of implantation.

35

Western blot analysis confirmed the presence
of a cytokeratin protein associated with rabbit
bladder urothelium in implants recovered after 30
days. This results suggests that some cell
5 type-specific functions associated with bladder
differentiation may be retained in implants after
extended periods. We have also presented an
efficient method of uroepithelial cell harvest which
will allow the efficacy of reconstitution of
10 urothelial cell growth, structure and function to be
studied more extensively in this model system.

Reconstructive procedures involving the
bladder, ureters, urethra and kidney frequently
15 employ bowel segments, despite the potential for
numerous complications. The benefit of using bladder
mucosa in these procedures is self-evident, however a
suitable urothelial substitute for bowel would
require the creation of autologous urothelial sheets,
20 most likely originating from limited biopsy
material. Harvesting and expansion of autologous
urothelium, in the manner successfully employed with
skin, may not be possible, due to the relatively
limited proliferative potential of differentiated
25 uroepithelium in vitro. This limitation may be
overcome by use of molecular regulators of
uroepithelial growth and differentiation.

The present study provides evidence that the
creation of urothelial structures de novo from
diassociated urothelial cells can be achieved in vivo
using biodegradable polymers as delivery vehicles.
5 Unwoven polyglycolic acid polymers provided an
adequate surface for urothelial cell attachment and
were found to serve as suitable substrates for
urothelial survival, and possibly growth, in vivo.
Further, RUC on these polymers were found to orient
10 themselves spatially in the host animals, and to
exhibit some aspects of urothelial cell
differentiation (cytokeratin expression), even after
extended periods. Significantly, contiguous layers
of presumptive uroepithelium were identified in most
15 of the RUC-polymer implants found to contain
eosinophilic cells, even though the cell harvest
procedure involved the isolation and seeding of
suspensions of single cells. This indicates that
these polymers can support the spontaneous
20 reorientation of uroepithelium into layered
structures resembling normal transitional cell
architecture.

Some differentiated cell types, such as
25 chondrocytes and hepatocytes, have been found to
remain functionally differentiated and in some cases
to expand in vivo on nonwoven polyglycolic acid or
polylactic acid polymers. The polymer fibers provide
sites for cell attachment, the reticular nature of
30 the polymer lattice allows for gas exchange to occur
over considerably less than limiting distances, and
the polymers evoke host cell responses, such as
angiogenesis which promote cell growth.

Synthetic polymers can also be modified in
vitro before use, and can carry growth factors and
other physiologic agents such as peptide and steroid
hormones, which promote proliferation and
5 differentiation. The malleability of the synthetic
polymer used in this study also should allow for the
creation of cell-polymer implants manipulated into
preformed configurations (e.g., tubes in the case of
urothelium).
10

In genitourinary reconstruction,
cell-polymer allografts can be implanted along
existing urinary structures in manipulated forms
resembling normal in vivo architecture. The
15 polyglycolic acid polymer undergoes biodegradation
over a four month period; therefore as a cell
delivery vehicle it permits the gross form of the
tissue structure to be reconstituted in vitro before
implantation with subsequent replacement of the
20 polymer by an expanding population of engrafted cells.

The newly-developed urinary structure can
thereby mimic the gross configuration of the polymer
scaffold, be composed of transplanted uroepithelial
25 cells and their derivatives, be functional in situ,
and possibly be translatable to another site in a
reconstructive procedure.

Although this invention has been described
with reference to specific embodiments, variations
and modifications of the method and means for
constructing urothelial implants by culturing
5 urothelial cells on matrices having maximized surface
area and exposure to the surrounding
nutrient-containing environment will be apparent to
those skilled in the art. Such modifications and
variations are intended to come within the scope of
10 the appended claims.

We claim:

1.   A system for growing a prosthetic urological structure comprising:

a biocompatible, biodegradable polymeric matrix in a nutrient environment; and

5   urothelial cells attached the matrix, wherein the matrix is structured to provide free exchange of nutrients and waste to attached cells in the absence of vascularization.

10   2.   The system of claim 1 wherein the polymer is selected from the group consisting of polyanhydride, polyorthoester, polyglycolic acid, polylactic acid and combinations thereof.

15   3.   The system of claim 1 further comprising coatings on the polymer selected from the group consisting of basement membrane components, agar, agarose, gelatin, gum arabic, collagens, fibronectin, laminin, glycosaminoglycans and mixtures

20 thereof.

4.   The system of claim 1 wherein the matrix forms a flexible structure conformable to biological surface.

25

5.   The system of claim 1 wherein the matrix is formed of fibers.

6.   A method for making a chimeric

30 urological structure comprising:

providing a biocompatible, biodegradable polymeric matrix in a nutrient environment and

attaching urothelial cells to the

35 matrix to form a hybrid structure.

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    7.   The method of claim 6 wherein the
polymer is selected from the group consisting of
polyanhydride, polyorthoester and polyglycolic acid.

5   8.   The method of claim 6 further
comprising coating the polymer with a material
selected from the group consisting of basement
membrane components, agar, agarose, gelatin, gum
arabic, collagens, fibronectin, laminin,
10 glycosaminoglycans, attachment peptides, functional
equivalents, and mixtures thereof.

    9.   The method of claim 6 wherein the
matrix is formed as a flexible structure conformable
15 to a biological surface.

    10.   The method of claim 6 wherein the
nutrient environment is _in vivo_ further comprising
attaching the cells on a matrix and implanting the
20 attached cells on the matrix in an animal without
first proliferating the cells on the matrix _in vitro_.

    11.   The method of claim 6 further
comprising attaching the cells on a matrix,
25 proliferating the cells on the matrix _in vitro_ in a
nutrient media, then implanting the cells on the
matrix _in vivo_.

    12.   The method of claim 6 further
30 comprising implanting the structure to form a
urological prosthesis.

13.   A method isolating urothelial cells for
culturing purposes, the method comprising:
          exposing urological tissue to an enzyme
solution; and
5   collecting urothelial cells which are
dissociated by said solution.

14.   The method of claim 13 wherein the step
of exposing said tissue to an enzyme solution further
10 comprises exposing said tissue to a collagenase
solution.

15.   The method of claim 13 wherein the
concentration of the solution ranges from about 0.05
15 percent to about 0.75 percent collagenase.

16.   The method of claim 13 wherein the step
of exposing said tissue further comprises excising a
tissue biopsy, isolating the mucosa from the biopsy
20 and placing the mucosa in said solution.

17.   The method of claim 13 wherein the step
of exposing said tissue further comprises irrigating
the interior of a bladder in vivo with said solution.
25

18.   The method of claim 13 wherein the step
of collecting urothelial cells further comprises
centrifuging a solution containing the dissociated
cells.
30

19. The method of claim 13 wherein the method further comprises:

providing a biocompatible, biodegradable polymeric matrix in a nutrient
5 environment and

attaching urothelial cells to the matrix to form a hybrid structure.

20. The method of claim 19 wherein the
10 method further comprises culturing the cells and matrix together to create a urological structure and then implanting the structure to form a urological prosthesis.

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61L27/00;   C12N5/00;   A61F2/06

II. FIELDS SEARCHED

Minimum Documentation Searched[7]	
Classification System	Classification Symbols
Int.Cl. 5	A61L ; C12N ; A61F

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category°	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	WO,A,9 012 604 (J. VACANTI) 1 November 1990 see page 16, line 26 - page 27, line 2; claims see page 10, line 30 - page 16, line 2 ---	1-9, 13-15, 18-19
Y	WO,A,8 803 785 (J. VACANTI) 2 June 1988 see page 10 - page 17; claims & US PATENT APPLICATION 933018 (20-11-1986) cited in the application ---	1-9, 13-15, 18-19
	-/--	

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28 JANUARY 1993	9. 02. 93
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	G.COUSINS- VAN STEEN

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category°	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	THE JOURNAL OF UROLOGY vol. 133, no. 5, May 1985, pages 866 - 869 TACHIBANA E.A. 'URETERAL REPLACEMENT USING COLLAGEN SPONGE TUBE GRAFTS' see the whole document	1-9, 13-15, 18-19
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P,X	THE JOURNAL OF UROLOGY vol. 148, no. 2PT2, August 1992, pages 658 - 662 ATALA E.A. 'FORMATION OF UROTHELIAL STRUCTURES IN VIVO FROM DISSOCIATED CELLS ATTACHED TO BIODEGRADABLE POLYMER SCAFFOLDS IN VITRO'	1-9, 13-15, 18-19

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The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.   28/01/93

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WO-A-8803785	02-06-88	EP-A- 0299010 JP-T- 1501362 US-A- 5041138	18-01-89 18-05-89 20-08-91
US-A-4645669	24-02-87	None	

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82